Effect of fipronil seed treatments on the germination and early growth of rice

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Abstract: Fipronil seed treatments were evaluated to determine whether they directly influence germination and subsequent seedling growth in rice (*Oryza sativa* L). Continuous seed exposure to fipronil (four days) at 2000 mg litre^{-1} significantly impaired germination ($P<0.001$). When exposure was restricted to a 1-h period 48 h after the initiation of germination, early post-germination growth was also impaired (assessment two days after exposure, $P<0.05$). The proportion of seeds satisfying our criteria for normal germination fell by 2.3 and 2.6% respectively across 17 cultivars. Cultivar effects were highly significant ($P<0.001$). When exposure to fipronil (2000 mg litre^{-1}) was restricted to 2 h at initial seed wetting no significant growth impairment occurred. No significant differences ($P>0.05$) were found between shoot lengths or root system dry weights of control plants and plants developing from seed exposed continuously (two days) to fipronil at rates of up to 2000 mg litre^{-1} during germination and harvested nine days after sowing. Treating germinated seed with fipronil for 1 h immediately prior to sowing at rates of up to 4000 mg litre^{-1} did not result in significant changes ($P<0.05$) in plant growth parameters at either nine or 25 days after sowing. No evidence of fipronil having a direct phytostimulatory effect on rice was obtained.

Keywords: fipronil; rice; germination; phytotoxicity

1 INTRODUCTION

Seed treatments are a convenient and effective method of protecting crops against pests and diseases. One of the main prerequisites for their use is that they must not have substantial adverse effects on seed germination and subsequent plant growth.[1,2] In wet-seeded rice, the use of seed treatments can be particularly advantageous to growers, as it may allow a reduction in the aerial spraying of pesticides, reducing both production costs and the risk of off-target spray drift. Seed treatments are commonly used to protect wet-seeded rice against pests and diseases,[1,3] and have also been evaluated as a means of delivering herbicides into the crop;[4] however, specific fungicide, insecticide, and herbicide seed treatments may be phytotoxic to rice.[4–6] Different stereoisomers of individual compounds may also vary in their level of phytotoxicity.[7] Careful screening of seed treatments is therefore essential prior to their commercial use.

The phenyl pyrazole insecticide fipronil is currently being developed for the control of a large number of crop pests, including pests of rice. In the USA, fipronil has been shown to provide effective control of rice water weevil, *Lissorhoptrus oryzophilus* Kuschel,[8–11] whilst in Australia fipronil is an effective seed treatment for the control of chironomid midge larvae, including those of the rice bloodworm, *Chironomus tepperi* Skuse.[12] Applying fipronil to germinated seed prior to sowing at rates as low as 12.5 g AI ha^{-1} is an effective technique for chironomid control,[12] whilst seed treatments have also been shown to be effective for controlling rice water weevil.[9,10]

Rice *et al*,[10] in a study on the efficacy of fipronil for the control of *L. oryzophilus*, found significant increases in plant height in fipronil-treated field plots, and suggested that fipronil may have a stimulatory effect on plant growth. Stevens *et al*[12] found fipronil to be a superior seed treatment to malathion for chironomid control, and reported better establishment, increased shoot lengths, and better root system dry weights in field plots. The residual control provided by the fipronil treatments, whilst superior to malathion, did not appear to explain these factors adequately, leading to the conclusion that fipronil may stimulate plant growth, or alternatively have a systemic effect. The residual efficacy of a systemic seed treatment could be underestimated in a situation where assessments are based purely on euryphagous larval populations, since the larvae may recolonise fields after treatment and utilise other food sources, such as decaying organic

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matter, in response to the ongoing systemic activity within the rice plants.

Accurately determining the effect of insecticidal seed treatments on plant growth in field efficacy trials is complicated by the presence of pests, which makes it difficult to determine whether differences in plant growth are a direct consequence of either a phytotoxic or phytostimulatory response to the treatment itself, or an indirect consequence of differences in the insecticidal effectiveness of the treatment relative to the standard against which comparisons are being made. This study was conducted under laboratory conditions and in the absence of pests in order to determine whether fipronil seed treatments affect the germination and early growth of selected Australian, Asian and American rice cultivars.

2 MATERIALS AND METHODS

Fipronil was used as a 500 g litre^{-1} suspension concentrate. (Cosmos®; RP EXP 80415A; Rhône-Poulenc).

Six separate experiments were conducted to assess the influence of fipronil seed treatments on seed germination and early seedling growth. Within each experiment, seed of individual cultivars was taken from a common sample stored at 4 (±1) °C.

2.1 Germination impairment – continuous and short-term initial exposure of seed to fipronil

2.1.1 Experiment 1. Continuous exposure of seed

Paired samples (100 seeds each) of each of 17 selected rice (*Oryza sativa* L) cultivars (11 Australian, three American and three Asian) were placed in flat-bottomed glass tubes (25 mm internal diameter). Fipronil 500 g litre^{-1} SC was diluted in distilled water to 2000 mg AI litre^{-1}, and was added (15 ml) to one tube of each pair, whilst control tubes were treated with an identical volume of distilled water. All tubes (including controls) were capped, maintained at room temperature (*c* 23 °C), and inverted and agitated (30 s) every 30 min for 2 h. Seed samples and the supernatant solutions were then transferred to glass Petri dishes (90 mm diameter) containing a single filter paper (Whatman No 1). Lids were placed on the dishes and they were maintained in darkness (30(±1) °C) for four days until assessment. Germination was considered to have been impaired if neither the radicle nor coleoptile was ≥1 mm in length.

2.1.2 Experiment 2. Short-term initial exposure of seed

This experiment was conducted using the same basic procedure as experiment 1, but after 2 h immersion in either fipronil (2000 mg AI litre^{-1}) or distilled water (*c* 23 °C, 30 s agitation every 30 min) the seed samples (including controls) were transferred to 25-mm internal diameter perspex tubes with wire-mesh bottoms. These were mounted under a hose nozzle, allowing each sample to be flushed with tap water (10 min). The seed samples were then transferred into glass Petri dishes (90 mm diameter) containing distilled water (15 ml) and a single filter paper. Maintenance conditions and assessment criteria were as for experiment 1.

2.2 Short-term exposure of seed to fipronil after germination

Experiment 3 was designed to simulate the proposed seed treatment procedure for the commercial use of fipronil on NSW rice farms, where seed is germinated over a 48-h period and treated with fipronil as it is augered into loaders for transferral to aircraft. Samples of 100 seeds were prepared in pairs and distilled water (15 ml) was added to each tube. Tubes were maintained in darkness at 30(±1) °C for 24 h, and the excess water was drained off. After a further 24 h under the same conditions, treatment samples were soaked in 2000 mg AI litre^{-1} fipronil for 1 h, whilst control samples were soaked in distilled water (*c* 23 °C, 30 s agitation every 15 min). The seed samples were rinsed prior to transfer to Petri dishes (as for experiment 2), and were then maintained in darkness (30(±1) °C) for a further two days before assessment. As the seed had already germinated before exposure to fipronil began, an alternative assessment criterion was used. Early plant growth was considered to have been impaired if neither the radicle nor coleoptile was ≥3 mm in length.

Experiments 1–3 were replicated four times for each of the 17 cultivars, ie, a total of 13 600 seeds were used per experiment.

2.3 Data analysis for experiments 1 to 3

A generalised linear model incorporating a binomial distribution with logit-link function[13] was used to assess the significance of treatments, cultivars, and their interaction within each experiment. The initially fitted terms were;

$$\text{constant} + \text{cultivar} + \text{treatment}$$

For graphical representation, treatment data were individually corrected against controls using Abbott's formula[14] prior to the calculation of means and standard errors for each cultivar.

2.4 Effects on plant growth at nine and 25 days after sowing

2.4.1 Experiment 4. Continuous exposure of seed to fipronil during germination, assessment nine days after sowing

Four cultivars were examined (country of origin in parentheses), *Millin, Kyeema* (Australia), *Koshihikari* (Japan), and *Lemont* (USA).

Five samples (8 g each) of dry seed were weighed into glass tubes (32 mm internal diameter). Fipronil was diluted with distilled water to give concentrations of 250, 500, 1000 and 2000 mg AI litre^{-1}, and one of these suspensions (25 ml) was added to each of the four treatment samples. The control sample was treated with distilled water (25 ml). Samples were agitated (30 s) at 30-min intervals for 2 h at room temperatures (*c* 23 °C), and then maintained in darkness (30(±1) °C) for 24 h, prior to draining off

the supernatant. After a further 24 h under the same conditions 18 seeds from each sample were selected for sowing.

Topsoil from a Birganbigil clay/loam[15] was milled, autoclaved (105 °C, 1 h.) and placed in a layer (30 mm) in the bottom of five translucent HDPE trays, each 155 × 102 × 70 mm (height). Trays were flooded to a depth of 20 mm above soil level with 1 × Martins rearing solution,[16] a salt solution containing 75 mg litre^{-1} total dissolved solids that had previously been found effective for rearing rice plants in experiments involving assessment of chironomid midge damage (Stevens MM, unpublished). The trays were then placed in illuminated seed germination cabinets (Thermoline Pty Ltd, Sydney). Eighteen seeds per container were placed on the soil/water interface in three evenly spaced rows and an identical HDPE tray was then inverted over the bottom tray to diffuse the light and reduce evaporation. Upper and lower trays were secured together using clear adhesive tape. Aeration to the solution in each tray was provided by a hypodermic needle attached to an aquarium aerator. Trays were maintained at 28 °/20 °C (12:12 cycle, 15L:9D illumination) for nine days prior to harvesting.

Plants were carefully removed from the soil and shoot lengths were measured to the nearest 0.5 mm. Root systems were separated and dried individually to constant weight at 105 °C prior to weighing on an analytical balance. Three replicates (90 plants each) were conducted for each cultivar. Analyses were undertaken using ANOVA and least significant difference procedures. Results for shoot lengths and root system dry weights were analysed separately for each cultivar, whilst a combined analysis of all cultivars was also conducted. In all cases container means were analysed, with individual plants being considered as sub-sample units.

2.4.2 Experiment 5. Short-term exposure of seed to fipronil after germination, assessment nine days after sowing

Experiment 5 was similar to experiment 4, however seed samples were soaked in distilled water (25 ml) for 24 h (30 ± 1 °C in darkness), the supernatant was drained away, and the seed was then maintained for a further 24 h under the same conditions. Fipronil suspension at 500, 1 000, 2 000 or 4 000 mg AI litre^{-1} or distilled water (25 ml) was then added. Samples were agitated (30 s) at 15-min intervals for 1 h at room temperature (*c* 23 °C) prior to sowing (18 seeds per container). Assessments were made nine days after sowing using the same procedure as in experiment 4. Three replicates (90 plants each) were conducted for each of the four cultivars. Statistical analysis was as for experiment 4.

2.4.3 Experiment 6. Short-term exposure of seed to fipronil after germination, assessment 25 days after sowing

Two cultivars (*Millin* and *Kyeema*) were assessed separately. Seed preparation and treatment rates were as for experiment 5. Forty trays (prepared as for experiment 5) were used for each replicate of each cultivar. For each treatment, eight seeds were placed on the soil/water interface (two evenly spaced rows) in each of eight trays. Trays were labelled, randomised, and placed in a controlled-temperature room at 28 °/20 °C, (12:12 cycle, 15L:9D illumination) under horizontal fluorescent light banks. The trays were not covered and supplementary aeration was not provided. Trays were topped up daily with distilled water to compensate for evaporation. Assessments of shoot lengths and root dry weights were made 25 days after sowing using the same procedure as in experiments 4 and 5. Two replicates (320 plants each) were conducted per cultivar. Results (container means) for each cultivar were analysed using ANOVA and least significant difference procedures.

3 RESULTS

3.1 Germination impairment – continuous and short-term initial exposure of seed to fipronil

Results for experiments 1 and 2 are shown in Fig 1(a,b). When exposed continuously to fipronil at 2 000 mg AI litre^{-1}, normal germination was significantly ($P < 0.001$) reduced (Fig 1a). Cultivar and treatment effects were both significant, but their interactions were not. The average fall in normal germination across all cultivars was 2.3% (range of effects 8.0% decrease to 0.8% increase). When fipronil exposure (2 000 mg AI litre^{-1}) was limited to an initial 2-h period there was no significant treatment effect ($P > 0.05$, Fig 1b). Cultivar effects were again significant ($P < 0.001$), but the interactions between cultivars and treatments were not ($P > 0.05$). An average increase in normal germination across all cultivars of 0.6% (range of effects 3.9% decrease to 7.7% increase) was recorded.

3.2 Short-term exposure of seed to fipronil after germination

Results for experiment 3 are shown in Fig 1c. A 1-h exposure of germinated seed to fipronil (2 000 mg AI litre^{-1}) significantly lowered the number of plants meeting the criterion for normal early growth ($P < 0.05$); however the mean reduction across all cultivars was only 2.6% (range of effects 12.0% decrease to 6.8% increase). Cultivar effects were highly significant ($P < 0.001$), but the interaction between treatments and cultivars was not ($P > 0.05$).

3.3 Effects on plant growth at nine and 25 days after sowing

3.3.1 Experiment 4. Continuous exposure of seed to fipronil during germination, assessment nine days after sowing

Results from experiment 4 are shown in Table 1. Continuous exposure of seed to fipronil during germination at rates between 250 and 2000 mg AI litre^{-1} did not significantly affect root or shoot development in any of the four cultivars tested ($P > 0.05$). Changes in shoot length (relative to

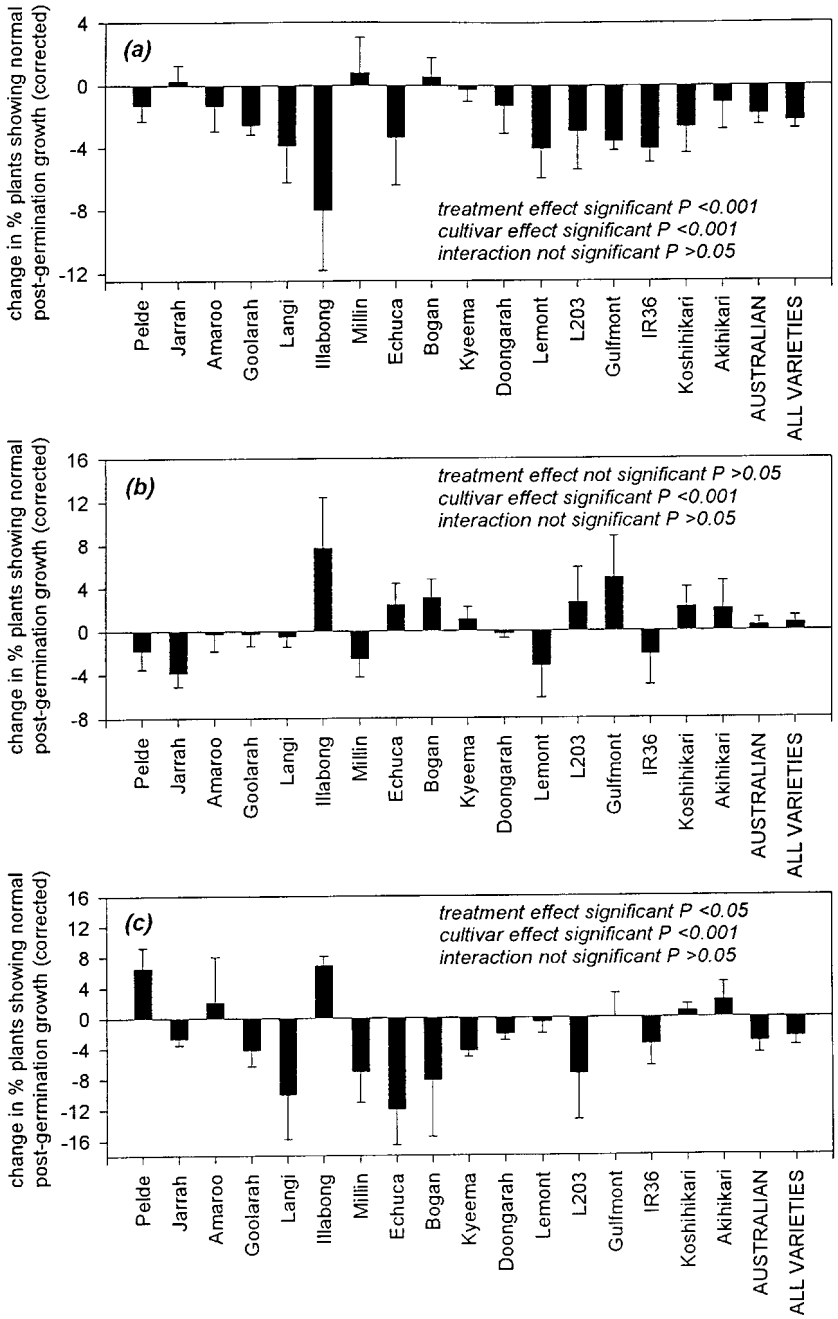

Figure 1. Influence of fipronil seed treatment (2000 mg AI litre^{-1}) on germination and subsequent early growth of rice. (a), experiment 1, continuous exposure, (b) experiment 2, 2 h exposure at initial seed wetting, (c), experiment 3, 1 h exposure after germination. Error bars represent standard errors.

controls within individual cultivars) varied between a 19.6% increase and a 13.9% decrease (mean: 3.1% increase), whilst changes in root system dry weights varied between a 15.4% increase and a 7.1% decrease (mean: 1.1% increase). The combined analysis of data across all cultivars showed that only cultivar effects were significant ($P<0.001$); neither treatments nor the interactions between cultivars and treatments were significant ($P>0.05$).

3.3.2 Experiment 5. Short-term exposure of seed to fipronil after germination, assessment nine days after sowing

Results from experiment 5 are shown in Table 2. Short-term exposure of germinated seed to fipronil at concentrations between 500 and 4000 mg AI litre^{-1} immediately prior to sowing did not significantly affect root or shoot development in any of the four cultivars tested ($P>0.05$). Changes in shoot length (relative to controls within individual cultivars) varied between a 9.1% increase and a 19.1% decrease (mean: 4.1% decrease), whilst changes in root system dry weights varied between zero and a 22.9% decrease (mean: 11.8% decrease). The combined analysis of data across all cultivars again showed that only cultivar effects were significant ($P<0.001$); neither treatments nor the interactions between cultivars and treatments were significant ($P>0.05$).

3.3.3 Experiment 6. Short-term exposure of seed to fipronil after germination, assessment 25 days after sowing

Results from experiment 6 are shown in Fig 2. Short-term exposure of germinated seed to fipronil at concentrations between 500 and 4000 mg AI litre^{-1} immediately prior to sowing did not result in any

Shoot length (mm) (± SE)				
Treatment rate (mg litre^{-1})	*Millin*	*Kyeema*	*Lemont*	*Koshihikari*
0	145.8 (±9.0)	172.3 (±5.7)	94.8 (±4.2)	142.0 (±3.4)
250	158.1 (±14.6)	148.4 (±13.2)	96.3 (±2.4)	154.1 (±2.7)
500	145.9 (±9.4)	187.1 (±15.1)	109.2 (±9.4)	166.3 (±6.3)
1000	138.3 (±8.1)	166.9 (±7.8)	97.9 (±7.4)	151.7 (±8.9)
2000	131.8 (±11.8)	165.8 (±17.9)	90.7 (±7.5)	169.8 (±7.6)
Significance[a]	nsd	nsd	nsd	nsd
All cultivars combined: cultivar effects significant ($P<0.001$), treatment effect and interactions not significant ($P>0.05$)				
	Cultivar means			
(LSD 14.96)	144.0	168.1	97.8	156.8
Root system dry weights (mg) (± SE)				
Treatment rate (mg litre^{-1})	*Millin*	*Kyeema*	*Lemont*	*Koshihikari*
0	3.7 (±0.3)	2.1 (±0.2)	2.8 (±0.1)	2.6 (±0.1)
250	4.1 (±0.6)	2.2 (±0.2)	2.6 (±0.1)	2.6 (±0.2)
500	3.5 (±0.4)	2.4 (±0.1)	2.9 (±0.1)	3.0 (±0.1)
1000	3.5 (±0.3)	2.0 (±0.1)	2.9 (±0.3)	2.6 (±0.1)
2000	3.7 (±0.4)	2.0 (±0.3)	2.6 (±0.1)	2.6 (±0.1)
Significance[a]	nsd	nsd	nsd	nsd
All cultivars combined: cultivar effects significant ($P<0.001$), treatment effect and interactions not significant ($P>0.05$)				
	Cultivar means			
(LSD 0.3939)	3.719	2.152	2.782	2.695

[a] nsd = no significant difference between treatments ($P>0.05$).

Table 1. Experiment 4 results. Influence of continuous seed exposure to fipronil during germination, assessment nine days after sowing

significant differences ($P>0.05$) in shoot lengths or root system dry weights at 25 days after sowing.

4 DISCUSSION

Continuous exposure to formulated fipronil at 2000 mg AI litre^{-1} significantly ($P<0.001$) impaired rice seed germination, with 2.3% (mean of corrected cultivar means, experiment 1) of treated seeds failing to satisfy our criterion for 'normal' growth. When fipronil exposure was limited to the first 2 h of the germination process (experiment 2) no significant treatment effects were recorded; however when the seed was exposed to fipronil for 1 h after germination, treatment effects were again significant ($P<0.05$). The variable response to fipronil treatments shown in these experiments supports the hypothesis that fipronil uptake by rice seeds may be minimal during the early stages of seed soaking, and that significant uptake and subsequent growth inhibition may only occur if the exposure period occurs during, or extends into, the latter part of the germination process. Restricting seed exposure to the earliest stages of the germination process in order to avoid growth inhibition may be counterproductive, since potential systemic activity may be reduced by limiting fipronil uptake. Growth inhibition demonstrated in these experiments does not, however, represent 'germination failure', since the longer-term fate of seeds showing minimal or zero growth was not followed beyond the initial assessment periods.

Cultivar effects were highly significant ($P<0.001$) overall in experiments 1 to 3, although the significance of treatment effects on individual cultivars could not be accurately determined in these experiments due to the limited number of replicates and the generally low number of seeds failing to satisfy our criteria for normal growth. It should be noted that cv *Illabong*, which had poor control germination in experiments 1 to 3 (66% in comparison to 92% across the 16 other cultivars), showed the greatest variation in response to fipronil treatment, and the possibility that fipronil may have an even more pronounced effect on similar cultivars with inherently weak germination cannot be discounted.

Although a significant (2.6%, $P<0.05$) proportion of plants exposed to fipronil for 1 h after germination showed some inhibition of growth, this level of inhibition is considered too small to cause substantial problems with crop establishment in commercial

Shoot length (mm) (±SE)				
Treatment rate (mg litre^{-1})	Millin	Kyeema	Lemont	Koshihikari
0	167.4 (±9.4)	172.6 (±3.9)	115.0 (±4.0)	180.2 (±8.6)
500	148.6 (±3.7)	169.1 (±7.9)	109.9 (±8.4)	175.8 (±2.0)
1000	168.8 (±11.5)	176.1 (±11.0)	113.2 (±3.9)	151.2 (±11.9)
2000	162.0 (±1.2)	184.6 (±11.2)	121.2 (±7.6)	157.6 (±15.3)
4000	135.5 (±4.7)	188.3 (±3.5)	103.0 (±5.5)	168.1 (±11.0)
Significance[a]	nsd	nsd	nsd	nsd
All cultivars combined: cultivar effects significant ($P<0.001$), treatment effect and interactions not significant ($P>0.05$)				
Cultivar means				
(LSD 12.89)	156.5	178.1	112.5	166.6
Root system dry weights (mg) (±SE)				
Treatment rate (mg litre^{-1})	Millin	Kyeema	Lemont	Koshihikari
0	3.6 (±0.4)	2.4 (±0.3)	2.7 (±0.2)	3.5 (±0.5)
500	2.9 (±0.2)	2.1 (±0.1)	2.3 (±0.2)	3.5 (±0.6)
1000	3.4 (±0.1)	2.2 (±0.1)	2.2 (±0.1)	2.7 (±0.2)
2000	3.5 (±0.3)	2.3 (±0.2)	2.2 (±0.1)	3.0 (±0.3)
4000	3.1 (±0.3)	2.4 (±0.1)	2.2 (±0.1)	3.0 (±0.1)
Significance[a]	nsd	nsd	nsd	nsd
All cultivars combined: cultivar effects significant ($P<0.001$), treatment effect and interactions not significant ($P>0.05$)				
Cultivar means				
(LSD 0.4137)	3.301	2.264	2.310	3.132

[a] nsd = no significant difference between treatments ($P>0.05$).

Table 2. Experiment 5 results. Influence of short-term seed exposure to fipronil after germination, assessment nine days after sowing

situations. Although an apparent trend towards seedling growth inhibition, particularly of the root systems, was apparent nine days after sowing, no treatment effects were significant ($P<0.05$) and by 25 days after sowing neither significant treatment differences nor apparent trends could be discerned.

An apparent phytostimulatory response to fipronil in rice has been reported in the USA,[10] and in Australia Stevens *et al*[12] suggested that improved plant growth characteristics found in small plot trials could be a consequence of either a phytostimulatory response to fipronil or systemic activity. This study has provided no evidence for a phytostimulatory response to fipronil in rice, and the apparent 'en-

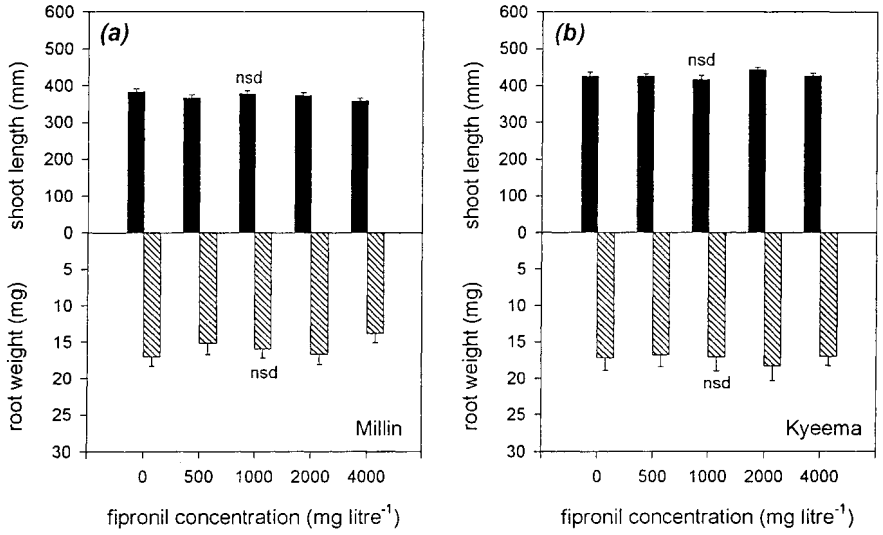

Figure 2. Influence of fipronil seed treatment (1h exposure after germination) on the subsequent growth of rice. Experiment 6, harvested 25 days after sowing. (a), cv *Millin*, (b) cv *Kyeema*. Error bars represent standard errors. nsd, no significant difference between treatments ($P>0.05$).

hanced growth' of fipronil-treated crops in the field remains unexplained. Further investigations are required to determine whether systemic activity is occurring, and whether enhanced residual control arising from such activity can account for differences between rice crops treated with fipronil and those treated with alternative compounds.

5 CONCLUSIONS

Fipronil seed treatments (2000 mg AI litre^{-1}) slightly but significantly inhibit the early growth of rice plants if the exposure period occurs partly or entirely after early seed wetting. Plants recover rapidly, with no significant differences to untreated controls nine days after sowing. Applying fipronil to germinated seed before sowing is unlikely to delay crop establishment substantially unless cultivars are unusually sensitive to the compound. No evidence was found to support a phytostimulatory response to fipronil in rice.

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